Recognition of *Biomphalaria* species (Gastropoda: Planorbidae) using the spiral growth variability, with description of a new fossil species

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ABSTRACT

The freshwater genus *Biomphalaria* Preston, 1910 is a well studied genus of freshwater snails, due to its importance as an intermediate host of the human parasite *Schistosoma mansoni* (Platyhelminthes). Soft parts are used to differentiate among modern species of *Biomphalaria*, but these parts are not present in fossils. The aim of this paper is to show another approach to discriminate among *Biompahalaria* species, by means of the analysis of the logarithmic spiral of the shell. We eompared five species, two modern and well known ones, *Biomphalaria peregrina* (d'Orbigny, 1835) and *Biomphalaria tenagophila* (d'Orbigny, 1835), a fossil one, *Biomphalaria walteri* (Parodiz, 1969), and two new fossil species. All fossils are from the Queguay Formation (Late Cretaeeous, Uruguay). The variance of the spiral was analized using a Kruskal-Wallis test with *a posteriori* Mann-Whitney pairwise comparisons. A morphological analysis had to be done to determine whether the specimens belong to different species. Our conclusions are that the analysis of the variance of the spiral coefficient can be used as a complementary character, but not as a primary method of differentiation between species. A new species is described as a result of the combined results of the statistical analysis and the traditional morphological description.

Additional Keywords: Late Cretaceous, logarithmic spiral, freshwater fossils

INTRODUCTION

Species of the freshwater gastropod *Biomphalaria* Preston, 1910 are distributed in the tropics and subtropics of the Americas and Africa (Taylor, 1988). Several species have medical importance because they are intermediate hosts of the human parasite *Schistosoma mansoni* (Platyhelminthes), whieh affects more than 200 million people in the world (WHO, 1985; Crompton et al., 1999; Chitsulo et al., 2000).

The shell in the genus is rather simple and not very informative taxonomically. Shell morphology of the genus, therefore, is used only as a secondary taxonomic criterion. Taxonomy of the genus is based on the anatomy, mainly the reproductive system (Paraense and Deslandes, 1959; Paraense, 1975, 1981, 1984, 1988; Luz et al., 1998), or even, and most recently, on molecular genetics, in particular DNA (Vidigal et al., 2000, 2004; Spatz et al., 1999; DeJong et al., 2001). A relatively recent review of the systematics of the genus can be found in Jarne et al. (2011).

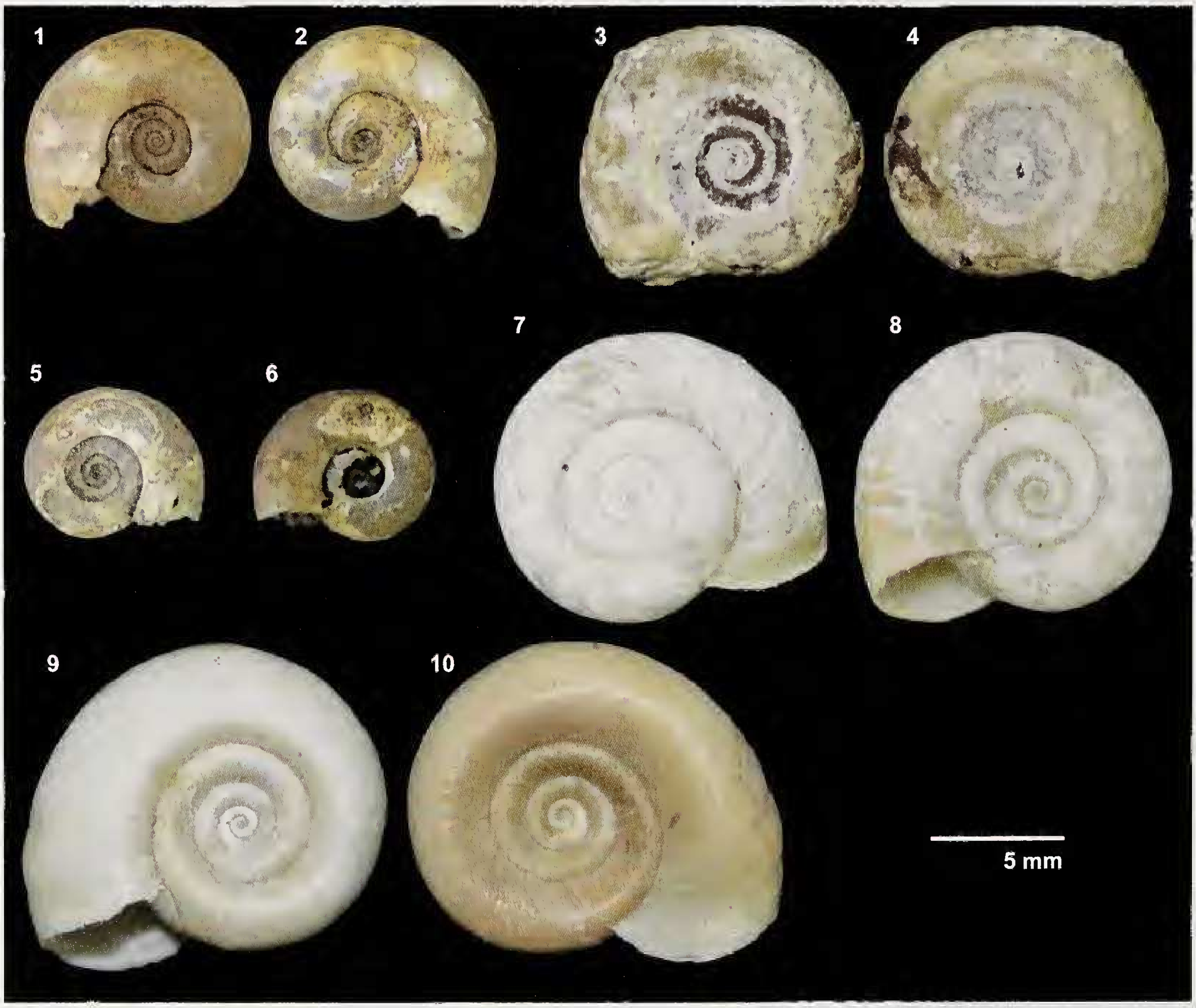
Some authors did statistical analyses of some shell dimensions (height, width), or number and shape of the whorls, but this methodology gave only approximate results (Jarne et al., 2011). Identification of species based only on the shell morphology is a common problem in mollusks, in particular in gastropods, and non-traditional morphometric solutions has been tried. For example, to distinguish between species of *Physa*, Samadi et al. (2000) studied shells and anatomical characters of some populations. They estimated Raup's (1966) parameters and analysed the variance. Unfortunately, the landmarks required in this method cannot be obtained for a planispiral shell, consequently the procedure is not useful for *Biomphalaria*. Johnston et al. (1991) modeled the spiral growth using geometric morphometrics, but they used shells of the marine genus *Epitonium* that has a conispiral shell with axial varices along the shell, and they could use the varix-suture intersections as landmarks. Determination of landmarks requires homologous intersections of lines, necessarily constant and repeatable in all specimens (Bookstein, 1991; Park et al. 2013). Due to its simple, planispiral shell, *Biomphalaria* does not have such intersections.

Therefore, neither Raup's parameters nor geometric morphometrics analyses can be done in shells of *Biomphalaria*. In this paper we tried a new approach for distinguishing speeies with planispiral shell. The shape of the spiral is a primary feature being used today to distinguish *a priori* different species of *Biomphalaria* (Bonetto et al., 1982, Johnston et al., 1991). In the present work, we quantify the spiral curve, in order to test its usefulness in taxonomic differentiation among several fossil and modern species.

Three fossil and two modern species were considered (Figures 1–10). Fossil shells of *Biomphalaria walteri* (Parodiz, 1969), and two fossil species assigned to *Biomphalaria*, one of them described at the conclusion of the paper as a new species, come from the limestones of Queguay Formation (Late Cretaceous) in Uruguay. One of the undetermined fossils was previously unknown (our *Biomphalaria* sp. 1), and another erroneously allocated to *Scolodonta semperi* Doering, 1874 by Morton and Herbst (1993). This latter species is herein described as new. The modern species used for the analysis were *B. peregrina* (d'Orbigny, 1835) and *B. tenagophila* (d'Orbigny, 1835). They currently inhabit Uruguay and surrounding regions.

MATERIALS AND METHODS

The three fossil specimens mentioned above, *Biomphalaria walteri* (Figures 1, 2), *Biomphalaria* sp. 1 (Figures 3, 4), and *Biomphalaria reversa* new species (Figures 5, 6) were compared with two recent species, *Biomphalaria peregrina* (Figures 7, 8) and *Biomphalaria tenagophila* (Figures 9, 10). The fossil specimens of *Biomphalaria walteri* and part of the specimens of *Biomphalaria* sp. 1 belong to the paleontological collection of Facultad de Ciencias (Montevideo, Uruguay) FCDP (4668, 6462, 6457, 6463, 6465); the rest of the specimens of *Biomphalaria* sp. 1 (no collection number) and the specimens of *Biomphalaria reversa* new species (PZ-CTES 5345) belong to the Paleontological Collection of Universidad Nacional del Nordeste (Corrientes, Argentina).



Figures 1–10. Shells of *Biomphalaria* species. **1–2.** *Biomphalaria walteri* (Parodiz, 1969); **3–4.** *Biomphalaria* sp. 1. **5–6.** *Biomphalaria reversa* new species, holotype. **7–8.** *Biomphalaria peregrina* (d'Orbigny, 1835). **9–10.** *Biomphalaria tenagophila* (d'Orbigny, 1835). For all species, the first figure is in apical view, second figure umbilical view. Seale bar=5 mm.

The recent and Quaternary specimens of *B. peregrina* and *B. tenagophila* are hosted in the collections of Museo Nacional de Historia Natural (MNHN, Montevideo, Uruguay, no collection number); Universidad Federal de Santa Maria (Santa Maria, Brazil) (BR100888, 101084, 101888, 100747); Paleontological Collection of Facultad de Ciencias, (FCDP 2097), and in a private collection (Gustavo Lecuona collection, GLC, without collection number). In total, 143 specimens were used in this work: 30 of *B. walteri*; 14 of *Biomphalaria* 1; 33 of *Biomphalaria reversa* new species; 36 of *B. tenagophila*, and 34 of *B. peregrina*.

The 143 specimens were photographed, digitized, and the spirals highlighted. Rectangles corresponding to each of the whorls of the spiral were plotted. Next, using the program Image Tool v. 3.00 (Wilcox et al., 2002), we measured height and width of the last three whorls of each of the shells from outside in (Figure 11). We used only the last three whorls because in most fossil specimens the initial whorls were broken or had encrustations.

The value of the logarithmic spiral coefficient (θ) was calculated as: $\theta \approx AB/AD$, AB being the width of a whorl, and AD the height; although this may not the exact value of θ, it may be a good approximation. The values of θ, θ′, and θ″ were obtained for each specimen, with AB/AD, $A'B'/A'D'$, and $A''B''/A''D''$ being the rectangles of the last three whorls (Figure 11). To obtain a unique value of θ per specimen the values were centered and standardized. Next, absolute θ values were converted to their natural logarithm to make them more comparable.

The selection of parametric or nonparametric analysis of variance (ANOVA) for analyzing the values of θ between species was determined via Shapiro-Wilk tests (SW) for normality of data and by Levene's tests (LV) for homogeneity of the variance. When deviations from normality and homogeneity were detected, a non-parametric Kruskal-Wallis test with *a posteriori* Mann-Whitney pairwise comparisons (Bonferroni corrected) test were applied (Sokal and Rohlf, 1998) to compare θ values of each species.

Then, height, width, and θ of each specimen were analyzed using Multivariate Analysis of Variance (MANOVA) and Canonical Variate Analysis (CVA) (Manly, 1994). The CVA produces a scatter-plot along the first canonical axis, showing the maximum separation between groups (multigroup discriminant analysis). The axes are a linear combination of the original values, and the eigenvalues indicate the variation of the axis and which variables contribute the most to discriminate the groups. The discriminating power of the variables was evaluated using Pillai trace because this may be more robust than Wilks lambda (Rencher, 2002). The relationship between width and height was analyzed using a linear regression model (Sokal and Rohlf, 1998), and the hypothesis of isometric growth ($b = 1$) was assessed applying Student t-test. PAST (v. 2.17c) (Hammer et al., 2001) was used for all statistical analysis. For all tests, the significance level used was $p=0.05$.

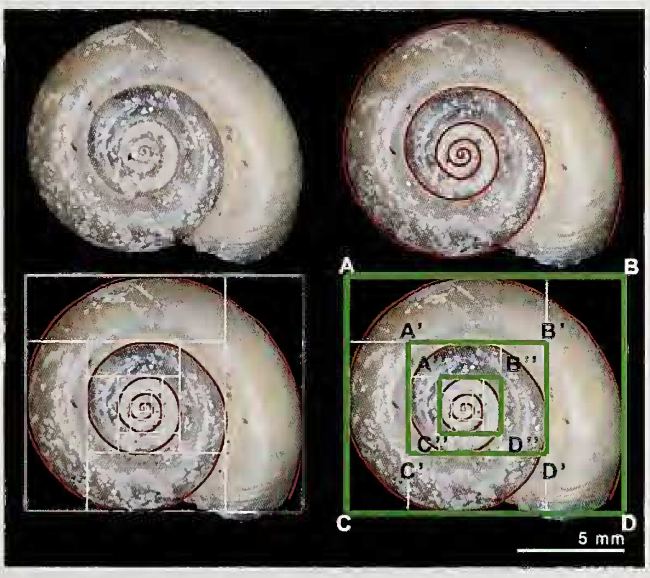


Figure 11. Step by step process to obtain θ values of the logarithmic spiral of the planispiral shell. θ can be approached by the ecuation $\theta \approx AB/AD$. The θ values of the three lasts whorls were averaged to obtain an unique value of θ for each specimen. Species: *Biomphalaria peregrina*. Scale bar=5 mm.

RESULTS

The regression model (Figure 12) between width and height was positive and significant (r = 0.99867, n=143,

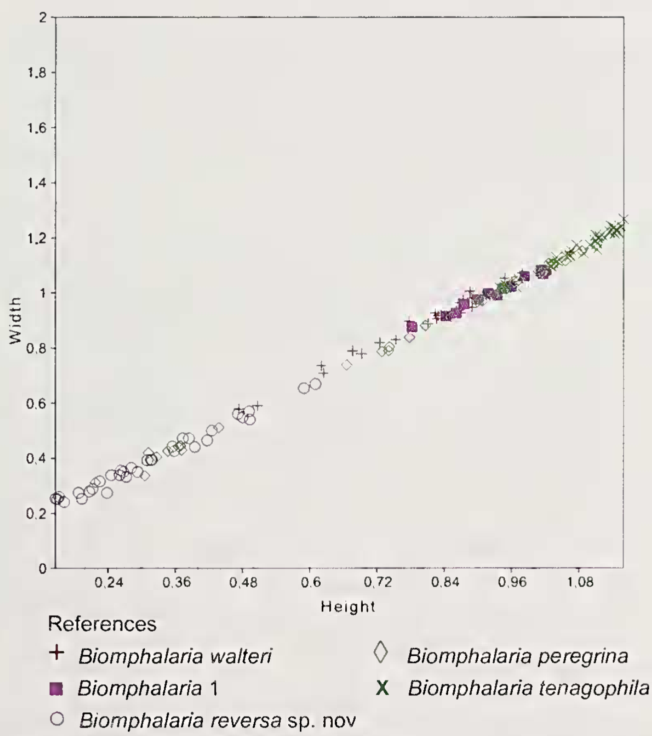


Figure 12. Regression model between width and height; r=0.99867, n=143, p<0.05, b=1.0008, s_b=0.0043523.

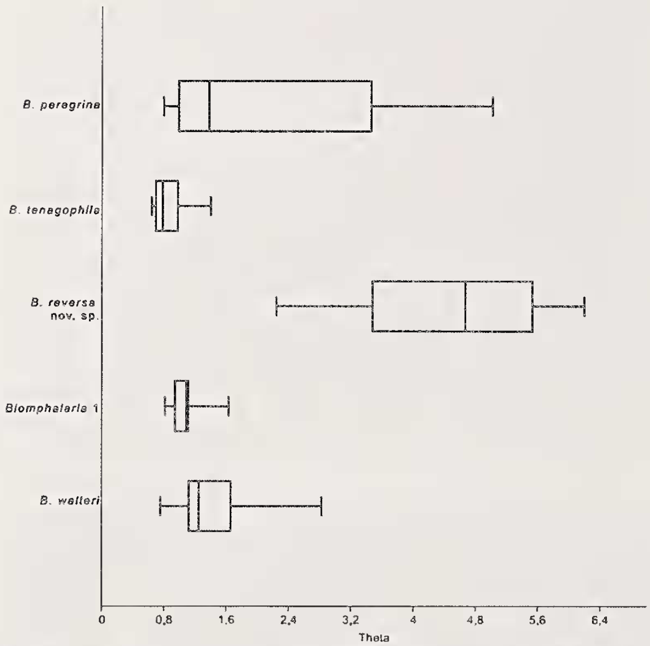


Figure 13. Box-plot of spiral coefficients (θ values) for each species. The box represents 25 and 75 percent quartiles, respectively. The median is shown with a horizontal line inside the box. The minimal and maximal values are shown with short horizontal lines ("whiskers").

$p < 0.05$) and showed an isometric shell growth when all species were considered (Figure 12) ($b=1.0008$, $s_b=0.0043523$ and $p=0.85528$); an allometric effect is therefore discarded.

A boxplot was performed to depict the basic statistical values of θ (Figure 13). The extant species *Biomphalaria peregrina* shows a greater dispersion than *B. tenagophila*, and this last one is bound to a restricted area of the graphic. Regarding the fossil species, *B. walteri* and *Biomphalaria* sp. 1 have almost the same dispersion, while *Biomphalaria reversa* new species is dispersed to the right of the chart and only shares part of the minimal values with the maximal values of *B. walteri*.

The θ data were non-normal (SW=0.94; p=9.64e–06), non-homogeneous among species (LV=9.8e-18; p=2.1e–38), and varied significantly among the analyzed species (Kruskall-Wallis, KW=98.52, p=2.034e–20). According to the Mann-Whitney (MW) *a posteriori* test with Bonferroni's correction, the modern species *B. tenagophila* and *B. peregrina* show differences between each other (p_{same}=1.879E–07). There is a similarity between the fossils *B. walteri* and *Biomphalaria* sp. 1 (p_{same}=0.053), and significant differences between *B. walteri* and *Biomphalaria reversa* new species (p_{same}=4.326E–10) and between *Biomphalaria* sp. 1 and *B. reversa* new species (p_{same}=8.234E–07). *Biomphalaria tenagophila* differs from all fossils as follows: *B. walteri* (p_{same}= 1.265E–07), *Biomphalaria* sp. 1 (p_{same}=0.00349), and *B. reversa* new species (p_{same}=1.008E–11). However, the fossils *B. walteri* and *Biomphalaria* sp. 1 are both similar to *B. peregrina* (p_{same}=1 with *B. walteri*, and p_{same}= 0.5528 with *Biomphalaria* sp. 1) (Table 1).

MANOVA Hotelling's p values (Bonferroni corrected) (Table 2) show significant differences between *Biomphalaria tenagophila* and *B. peregrina* (p=5.50382E–10); *B. walteri* shows differences with the remainder of the study species (p=0.0491237 with *Biomphalaria* sp. 1; p=2.35686E–16 with *Biomphalaria reversa* new species; p=4.02082E–06 with *B. tenagophila;* and p=0.000277502 with *B. peregrina*); *Biomphalaria* 1 shows differences with the other fossils but not with the extant species (p=2.69672E–12 with *Biomphalaria reversa* new species; p=0.0522732 with *B. tenagophila*, and p=0.107649 with *B. peregrina*); *Biomphalaria reversa* new species shows differences with all the species (p=1.50006E–24 with *B. tenagophila* and p=3.53252E–13 with *B. peregrina*). CVA was significant (Pillai trace=0.9918; F=17.53, p<<0.05). The first factor explains 89.55% of the total variability, and the

Table 1. Kruskal – Wallis test for θ values H (Chi^2) = 98.52; Hc (tie corrected) = 98.52; p_{same} = 2.034E-20. Mann-Whitney pairwise comparisons, Bonferroni corrected (below diagonal) \ uncorrected (above diagonal).

	B. walteri	*Biomphalaria sp. 1*	*B. reversa new species*	*B. tenagophila*	*B. peregrina*
B. walteri	0	0.0053	4.326E-11	1.265E-08	0.7183
Biomphalaria 1	0.053	0	8.234E-08	0.000349	0.05528
B. reversa nov. sp.	4.326E-10	8.234E-07	0	1.008E-12	7.133E-09
B. tenagophila	1.265E-07	0.00349	1.008E-11	0	1.879E-08
B. peregrina	1	0.5528	7.133E-08	1.879E-07	0

Table 2. MANOVA/CVA pairwise comparisons for width, height and θ. Hotelling's p values, uncorrected significance. It shows significant differences between most specimens (p-values<<α 0.05).

	B. walteri	*Biomphalaria sp. 1*	*B. reversa new species*	*B. peregrina*	*B. tenagophila*
B. walteri	0	0.0491237	2.35686E-16	4.02082E-06	0.000277502
B. sp. 1	0.0491237	0	2.69672E-12	0.0522732	0.107649
B. reversa new species	2.35686E-16	2.69672E-12	0	1.50006E-24	3.53252E-13
B. peregrina	4.02082E-06	0.0522732	1.50006E-24	0	5,50382E-10
B. tenagophila	0.000277502	0.107649	3.53252E-13	5.50382E-10	0

second the 6.2%. The variables that most contributed to distinguish among species groups were: height in the first factor and width in the second factor. CVA scatter-plot shows a well-separated *Biomphalaria reversa* new species in the left of the graphic, and *Biomphalaria* sp. 1 and *B. tenagophila* in the right. *Biomphalaria walteri* has a wide distribution in the right zone, and *B. peregrina* is distributed throughout the chart (Figure 14).

Analytical Discussion: The MW and MANOVA analyses for *Biomphalaria peregrina* and *B. tenagophila* show significant differences between the two species (Tables 1, 2). The CVA scatter-plot shows *B. peregrina* as having a wide distribution on the chart. This species shows large intraspecific variability, also recognized at the molecular level (Caldeira et al., 2001; Carvalho et al., 2001), and overlaps with the other extant species, *B. tenagophila*. Significant differenees for θ and for the other morphological variables suggest that with a simple shape as the logarithmic spiral of *Biomphalaria*, analyses of specimens of distinct species can yield similar shell shape results.

The statistical analysis of the extant species allows us to compare the statistical behavior of the fossils. *Biomphalaria walteri* and *Biomphalaria* sp. 1 do not show statistical differences, the MANOVA p-value for both is almost 0.05 (Table 2), and both show almost in the same area of the scatter plot, so there are no reasons to consider *Biomphalaria* sp. 1 as a different species from *B. walteri*. Regarding *B. reversa* new species, this fossil species presents significant differences with both *B. walteri* and *Biomphalaria* sp. 1. Moreover, in the scatter plot, the new fossil species presents a well-separated distribution in respect to the other two fossil species.

When the two extant species are compared with the fossils, θ value for *B. peregrina* is similar to the values for *B. walteri* and *Biomphalaria* sp. 1 and MANOVA test also showed similitudes to *Biomphalaria* 1 with the two extant species. *Biomphalaria reversa* new species showed significant differences with the two modern species in both statistical analysis. In the CVA scatter-plot, *B. walteri* and *Biomphalaria* 1 overlap with the two extant species, and *Biomphalaria reversa* new species overlaps with part of the distribution of *B. peregrina*.

The pairwise comparisons (MW) and the multivariate analysis of the variance (MANOVA) show that the spiral growths of *B. walteri* and *Biomphalaria* sp. 1 are almost coincident, and both differ from that of *Biomphalaria reversa* new species. When observing the results for distribution of multigroup discriminant analysis for the three fossils species in the CVA scatter plot, *Biomphalaria reversa* new species is well separated from the other two, and these overlap with each other. When the entire species set is observed in the CVA scatter plot, all of them can be considered as *B. peregrina*, so the scatter-plot by itself is not conclusive. We need to consider that *B. peregrina* is a species that shows a well-known, broad intraspecific variation, and as we used samples from Brazil and from Uruguay, perhaps regional variation is the reason for its wide distribution in the chart. Also, as mentioned before,

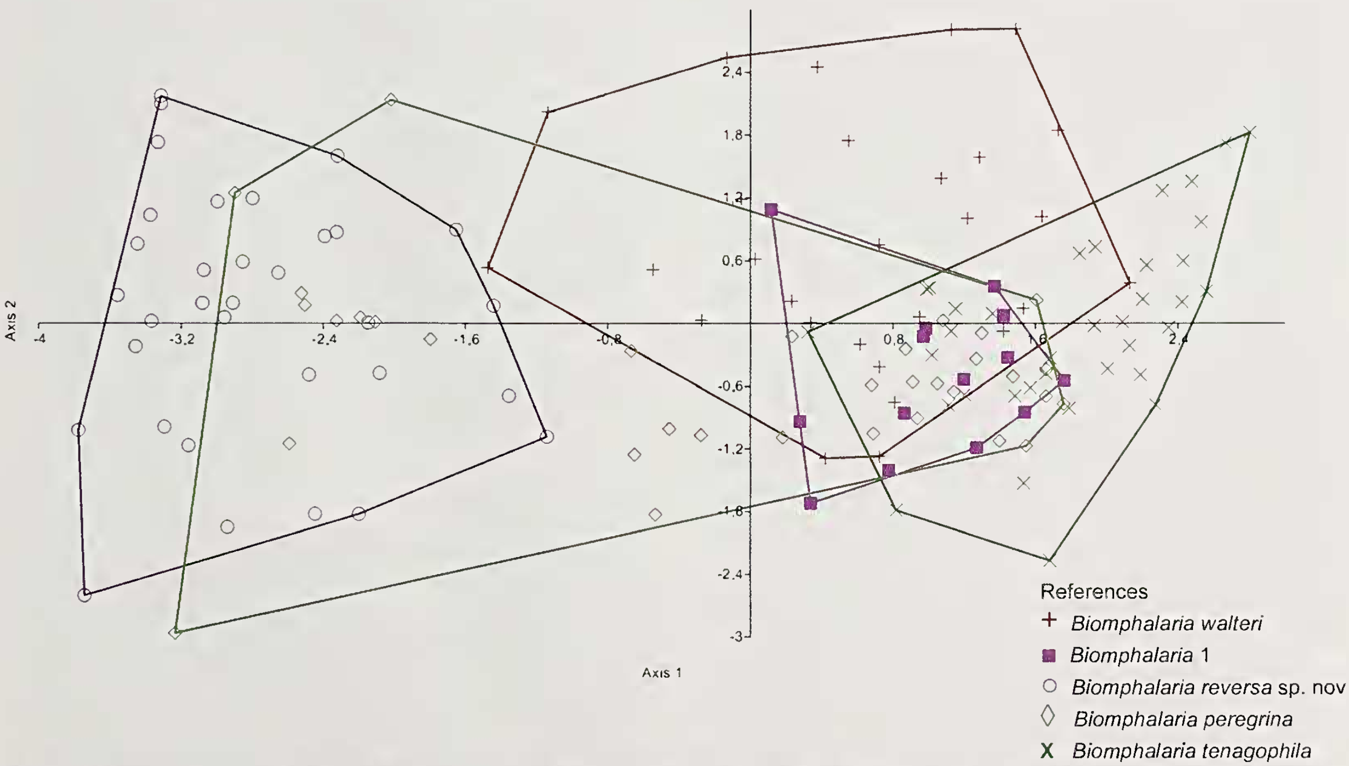


Figure 14. Canonical Variate Analysis (CVA) scatter plot for height, width and θ values for all the species.

it is expected that a simple shape as it is a logarithmic spiral could be repeated in the growth shape of different species.

To summarize, the statistical results show that the three variables explain the growth of each group as a whole. When it was expected that only θ would explain the variation, it turns out that the three variables together explain it. Hence, θ is a variable that is involved in the relative shell growth of each species, and not a variable that could explain the growth shape by itself. It is for that reason that the statistical discrimination may be complemented with qualitative morphological characteristics. This evidence leads to the formal description of a new species the new species as follows.

SYSTEMATICS

Superfamily Planorboidea
Family Planorbidae
Subfamily Planorbinae

Genus *Biomphalaria* Preston, 1910

Type Species: *Biomphalaria smithi* Preston, 1910 by monotypy.

***Biomphalaria reversa* new species**

(Figures 5, 6, 15–17)

Scolodonta semperi.—Morton and Herbst, 1993, p. 450, pl. I, figs. 9–10) (*non* Doering, 1874)

Diagnosis: Shell very small, sub-circular in apical view, oblong in apertural view; dextral spire with four whorls. In apical view, each whorl covers part of the previous whorl. Two last whorls visible in abapical view.

Description: Shell very small, sub-circular in apical view, planispiral, oblong in apertural view; dextral spire with four convex whorls. In apical view, early whorls closely stretched, being looser and wider in last portion of spire; last whorl is larger than preceding ones, covering partially previous whorl. In umbilical view, only last two whorls visible. Umbilicus present.

Type Material: Holotype: FCDPI 7230 (Figures 5, 6, 15–17), width 6.81, height 5.79, from type locality; Paratypes, three specimens, FCDPI 7830, segregated as 7830a (Figures 18–20); 7830b (Figures 21–23); and 7830c (Figures 24–26); all from Piedras Coloradas, Paysandú Department, Uruguay (Quegay Formation, Late Cretaceous)

Type Locality: Palmar, Soriano, Paysandú Department, Uruguay (Quegay Formation, Late Cretaceous).

Other Material Examined: FCDPI 7254, 7267, 7271 (4 specimens) Trinidad, Flores Department; 7220, 7253, 7259 (107 specimens) Piedras Coloradas, Paysandú Department; 7241, 7261, 7265, 7266, 7276, 7277 (78 specimens) Quebracho, Paysandú Department; 7038, 7039 (141 specimens) Nearby Algorta Town, Río Negro Department; 7829 (65 specimens) Palmar, Soriano Department; PZ-CTES 5345 (53 specimens) Piedras Coloradas, Paysandú Department (All Queguay Formation, Late Cretaceous).

Geographic and Stratigraphic Ranges: Nearby Algorta Town, Río Negro Department; Trinidad, Flores; Palmar, Soriano; Quebracho Town and proximities of Piedras Coloradas Town, Paysandú Department, Uruguay (All Queguay Formation, Late Cretaceous).

Etymology: Latin *reversa*, reverse, in referenee to the dextral shell orientation, in opposition to the sinistral orientation of the closest fossil species.

Comparative Remarks: The specimens allocated to *Scolodonta semperi* by Morton and Herbst (1993) represent *Biomphalaria reversa* new species. These specimens do not fit the description of *Scolodonta semperi* nor of any *Scolodonta* for that matter (Döring, 1875; Hausdorf, 2006). Species of *Scolodonta* have a discoidal shell with slowly increasing whorls and slightly raised spire (together with soft parts characters that are obviously only observable in recent species.) None of these characteristies are present in the fossil speeimens, their characters closely resembling those of *Biomphalaria* (see below).

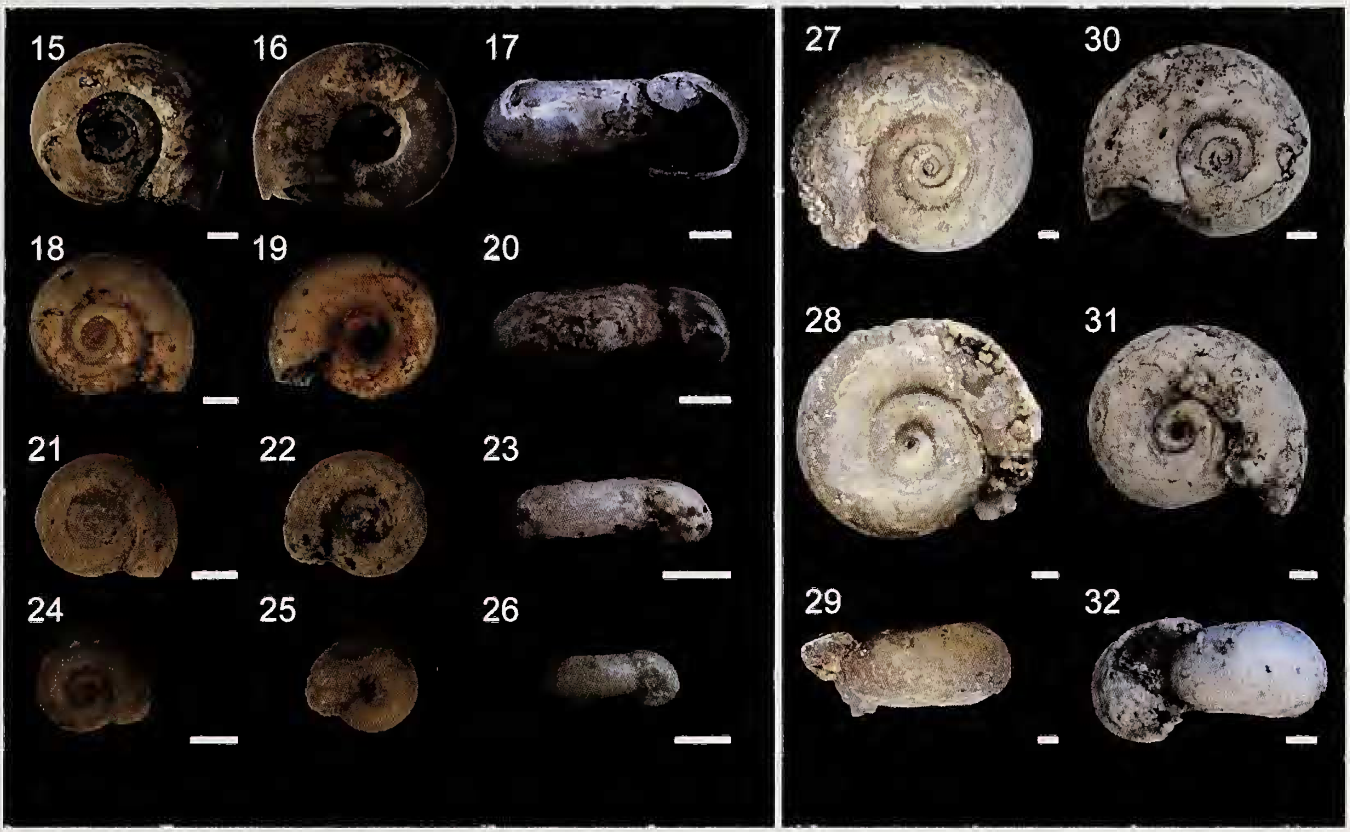
Biomphalaria reversa new species has its spiral growth very similar to *B. walteri*. However, it is clearly smaller than *B. walteri*. Additionally, *B. reversa* new species is dextral, and *B. walteri* is sinistral. *Biomphalaria reversa* has less whorls than *B. walteri* as well. In apertural view, *B. reversa* new species is more oblong and flattened than *B. walteri*.

The recent species *Biomphalaria peregrina* is similar to *B. reversa* new species in spiral growth and general shell shape (Figures 15–32). Additionally, both are dextral. However, *B. peregrina* is larger than *B. reversa* new species, and *B. peregrina* has more than six eircular whorls with well-defined sutures, while *B. reversa* has four slightly convex whorls, with barely visible sutures.

Biomphalaria tenagophila is larger than *Biomphalaria reversa* new species and has a sinistral spire with elevated and angled whorls, while *B. reversa* new species has "softer" and rounded whorls. The spiral coefficients showed significant differences between both species.

CONCLUSIONS

The analysis of the logarithmic spiral of the planispiral shells of the fossil and extant *Biomphalaria* species in this study, combined with shell width and height, describes the different spiral growth patterns in different species. Also, the analysis is useful as a complementary character for species identification, but not signifieant enough to be used by itself. Therefore, to make decisions about specimen identification, qualitative morphological characters had to be used. In fact, the quantitative



Figures 15–32. Shells of *Biomphalaria* species. **15–26.** *Biomphalaria reversa* new species, shells in apical, umbilical, and apertural view. **15–17.** Holotype (FCDPI 7230). **18–20.** Paratype a (FCDPI 7830a). **21–23.** Paratype b (FCDPI 7830b). **24-26.** Paratype c (FCDPI 7830c). **27–32.** *Biomphalaria walteri*, shells in apical, umbilical, and apertural view. **27–29.** Holotype, Carnegie Museum of Natural History, Pittsburgh (CM 103839) (courtesy of Dr. Timothy Pearce). **30–32.** FCDPI 6457. Scale bars: 1mm.

analysis and the traditional qualitative analysis complement each other in the definition of species in the genus *Biomphalaria*.

A new species is described as a result of the combined approaches of statistical analysis and traditional morphological description: *Biomphalaria reversa* new species, from the Queguay Formation (Late Cretaceous) of Uruguay. This new species is added to the freshwater assemblage of this lithostratigraphic unit, constituting, with *B. walteri,* the oldest representatives of *Biomphalaria* worldwide.

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LITERATURE CITED

Bonetto, A.A., J.A. Bechara, and M.P. Tassara. 1982. Los moluscos de la familia Planorbidae en el area del Río Paraná medio. Physis, Secc. B, 41(100): 1–6.

Bookstein, F.L. 1991. Morphometric Tools for Landmark Data: Geometry and Biology. Cambridge University Press, Cambridge Univ. Press, New York, 435 pp.

Caldeira, R.L., T.H.D.A. Vidigal, A.J.G. Simpson, and O.S. Carvalho. 2001. Genetic variability in Brazilian populations of *Biomphalaria straminea* complex detected by simple sequence repeat anchored polymerase chain reaction amplification. Memórias do Instituto Oswaldo Cruz 96: 535–544.

Carvalho, O.S., R.L. Caldeira, A.J.G. Simpson, and T.H.D.A. Vidigal. 2001. Genetic variability and molecular identification of Brazilian *Biomphalaria* species (Mollusca: Planorbidae). Parasitology 123: 197–209. doi:10.1017/S0031182001008058.

Chitsulo, L., D. Engels, A. Montresor, and L. Savioli. 2000. The global status of schistosomiasis and its control. Acta Tropica 77: 41–51.

Crompton, D.W.T. 1999. How much human helminthiasis is there in the world? Journal of Parasitology 85: 397–403.

DeJong, R.J., J.A.T. Morgan, W.L. Paraense, J-P. Pointier, M. Amarista, P.F.K. Ayeh-Kumi, A. Babiker, C.S. Barbosa, P. Brémond, A.P. Canese, C.P. De Souza, C. Dominguez, S. File, A. Gutierrez, R.N. Incani, T. Kawano, F. Kazibwe, J. Kpikpi, N.J.S. Lwambo, R. Mimpfoundi, F. Njiokou, J.N. Poda, M. Sene, L.E. Velásquez, M. Yong, C. M. Adema, B.V. Hofkin, G.M. Mkoji, and E.S. Loker. 2001. Evolutionary relationships and biogeography of *Biomphalaria* (Gastropoda: Planorbidae) with implications regarding its role as host of the human blood fluke, *Schistosoma mansoni*. Molecular Biology and Evolution 18: 2225–2239.

Döring, A. 1875. Apuntes sobre la Fauna de Moluscos de la Repúbliea Argentina (segunda parte). Boletín de la Academia Nacional de Ciencias Exactas de la Universidad de Córdoba 1: 432–460.

Hammer, O., D.A.T. Harper, and P.D. Ryan. 2001. PAST: Paleontological Statistics Software Package for Education and Data Analysis. Palaeontologia Electronica, 4(1), 9 pages. http://palaeo-electronica.org/2001_1/past/issue1_01.htm

Hausdorf, B. 2006. The systematic position of *Scolodonta* Döring, 1875 and Scolodontidae H. B. Baker, 1925 (Gastropoda: Pulmonata). Zoologischer Anzeiger 245: 161–165.

Jarne, P., J.P. Pointier and P. David. 2011. Biosystematics of *Biomphalaria* spp. with an Emphasis on *Biomphalaria glabrata*. In: R. and B. Fried (eds.) *Biomphalaria* Snails and Larval Trematodes. Springer, pp 1–32.

Johnston, M.R., R.E. Tabachnick and F.L. Bookstein. 1991. Landmark-based morphometrics of spiral accretionary growth. Paleobiology 17: 19–36.

Luz, E., S.M. Silva, A.P. de Carvalho and N. Castro. 1998. Atualização da sistemática e distribuição dos planorbídeos (Gastropoda, Pulmonata) no estado do Paraná (Brasil). Acta Biológica Paranaense 27: 39–55.

Manly, B.F.J. 1994. Multivariate statistical methods, a Primer. Chapman & Hall, London, 215 pp.

Morton, L.S. and R. Herbst. 1993. Gastrópodos del Cretácico (Formación Mercedes) del Uruguay. Ameghiniana 30: 445–452.

Paraense, W.L. 1975. Estado atual da sistemática dos planorbídeos brasileiros. Arquivos do Museu Nacional 55: 5–128.

Paraense, W.L. 1981. *Biomphalaria occidentalis* sp. n. from South America (Mollusca Basommatophora pulmonata). Memórias do Instituto Oswaldo Cruz 76: 199–211.

Paraense, W.L. 1984. *Biomphalaria tenagophila guaibensis* ssp. n. from Southern Brazil and Uruguay (Pulmonata: Planorbidae). I. Morphology. Memórias do Instituto Oswaldo Cruz 79: 465–469.

Paraense, W.L. 1988. *Biomphalaria kuhniana* (Clessin, 1883), planorbid mollusc from South America. Memórias do Instituto Oswaldo Cruz 83: 1–12.

Paraense, W.L. and N. Deslandes. 1959. The renal ridge as a reliable character for separating Taphius glabratus from *T. tenagophilus*. American Journal of Tropical Medicine and Hygiene 8: 456–472.

Park, P.J., W.E. Aguirre, D.A. Spikes, and J.M. Miyazaki. 2013. Landmark-based geometric morphometrics: what fish shapes can tell us about fish evolution. Tested Studies for Laboratory Teaching. Proceedings of the Association for Biology Laboratory Education 34: 361–371.

Parodiz, J.J. 1969. The Tertiary non-marine mollusca of South America. Annals of the Carnegie Museum 40: 1–242.

Preston, H.B. 1910. Additions to the non-Marine Molluscan Fauna of British and German East Africa and Lake Albert Edward. The Annals and Magazine of Natural History, Eighth Series, 35: 526–536.

Raup, D.M. 1966. Geometric analysis of shell coiling: general problems. Journal of Paleontology, 40: 1178–1190.

Rencher, A.C. 2002. Methods of multivariate analysis, 2nd ed. John Wiley & Sons, Inc., 727 pp.

Samadi, S., A. Roumegoux, M.D. Bargues, S. Mas-Coma, M. Yong, and J.-P. Pointier. 2000. Morphological studies of lymnaeid snails from the human fascioliasis endemic zone of Bolivia. Journal of Molluscan Studies 66: 31–44.

Sokal, R.R. and F.J. Rohlf. 1998. Biometry: the principles and practice of statistics in biological research, 2nd edition. W. H. Freeman and Company, New York, 859 pp.

Spatz, L., T.H.D.A. Vidigal, R.L. Caldeira, E. Dias Neto, S.M.G. Cappa, and O.S. Carvalho. 1999. Study of *Biomphalaria tenagophila*, *B. t. guaibensis* and *B. occidentalis* by polymerase chain reaction amplification and restriction enzyme digestion of the ribosomal RNA gene intergenic spacer. Journal of Molluscan Studies 65: 143–149.

Taylor, D.W. 1988. Aspects of freshwater mollusc ecological biogeography. Palaeogeography Palaeoclimatology Palaeoecology 62: 511–576.

Vidigal, T.H.D.A., R.L. Caldeira, A.J.G. Simpson, and O.S. Carvalho. 2000. Further studies on the molecular systematics of *Biomphalaria* snails from Brazil. Memórias do Instituto Oswaldo Cruz 95: 57–66.

Vidigal, T.H.D.A., L. Spatz, J.C. Kissinger, R.A.F. Redondo, E.C.R. Pires, A.J.G. Simpson, and O.S. Carvalho. 2004. Analysis of the first and second internal transcribed spacer sequences of the ribosomal DNA in *Biomphalaria tenagophila* complex (Mollusca: Planorbidae). Memórias do Instituto Oswaldo Cruz 99: 153–158.

WHO (World Health Organization). 1985. The Control of Schistosomiasis. WHOTechnical Series Report 728. WHO, Geneva.

Wilcox, D., B. Dove, D. Mcdavid, and D. Greer. 2002. Image Tool for Windows, v. 3. The University of Texas Health Science Center in San Antonio. http://ddsdx.uthscsa.edu/dig/itdesc.html.